(FILE 'HOME' ENTERED AT 15:01:40 ON 25 FEB 2003)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED AT 15:02:01 ON 25 FEB 2003

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L1             4 S 11D3 (L) ANTIBOD?
L2             1 DUP REM L1 (3 DUPLICATES REMOVED)
               E BRACCO LAURENT?/AU
L3             3 S E1
L4             3 DUP REM L3 (0 DUPLICATES REMOVED)
L5             3 SORT L4 PY
L6         11823 S P53 (L) ANTIBOD?
L7            44 S L6 AND (SINGLE CHAIN ANTIBOD?)
L8            20 DUP REM L7 (24 DUPLICATES REMOVED)
L9            20 SORT L8 PY

=> d an ti so au ab pi 19 1-3 5-8 12 14-19

L9   ANSWER 1 OF 20  CAPLUS  COPYRIGHT 2003 ACS
AN   1995:416390  CAPLUS
DN   122:308080
TI   Gene therapy for inhibition of intracellular processes by expression of
     antibody genes in the target cells
SO   Fr. Demande, 27 pp.
     CODEN: FRXXBL
IN   Schweighoifer, Fabien; Tocque, Bruno
AB   Antibodies, or other ligands for intracellular proteins, are manufd.
     intracellularly in target cells for inhibition of the function of a target
     protein.  This is achieved by cell-specific expression of the antibody
     gene using a replication-defective virus as the expression vector.  The
     coding sequence of the antibody gene (for a single chain
     antibody - ScFv) is modified to remove the secretion signals from
     the nascent protein.  The method is demonstrated by showing that
     intracellular expression of the gene for an ScFv against the HER-2 gene
     product lowered the effectiveness of Ha-Ras-mediated transformation of
     host cells .apprx.3-4-fold.
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	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	FR 2706486	A1	19941223	FR 1993-7241	19930616
	FR 2706486	B1	19950901		
	CA 2165458	AA	19941222	CA 1994-2165458	19940615
	WO 9429446	A2	19941222	WO 1994-FR714	19940615
	WO 9429446	A3	19950202		
	W: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, UZ, VN				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	AU 9470763	A1	19950103	AU 1994-70763	19940615
	EP 703980	A1	19960403	EP 1994-919711	19940615
	EP 703980	B1	20030129		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
	CN 1126491	A	19960710	CN 1994-192443	19940615
	CN 1076052	B	20011212		
	JP 08511162	T2	19961126	JP 1994-501434	19940615
	HU 74266	A2	19961128	HU 1995-3615	19940615
	HU 219138	B	20010228		
	BR 9407512	A	19970107	BR 1994-7512	19940615
	PL 180760	B1	20010430	PL 1994-312213	19940615
	CZ 289039	B6	20011017	CZ 1995-3295	19940615
	ZA 9404303	A	19950214	ZA 1994-4303	19940616
	NO 9505011	A	19951211	NO 1995-5011	19951211
	FI 9506057	A	19951215	FI 1995-6057	19951215
	US 6159947	A	20001212	US 1995-564164	19951228
	AU 9888403	A1	19981210	AU 1998-88403	19981009
	AU 722702	B2	20000810		

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L9   ANSWER 2 OF 20  CAPLUS  COPYRIGHT 2003 ACS
AN   1997:85151  CAPLUS
DN   126:85615
TI   Self-associating peptide domains for use in the formation of hetero- or
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homooligomeric proteins
SO PCT Int. Appl., 64 pp.
CODEN: PIXXD2
IN Pack, Peter; Hoess, Adolf
AB Self-assocg. peptides that can be used to direct the oligomerization of proteins into homo- or heterooligomers are described for use in the manuf. of oligomeric proteins by expression of cloned genes. These peptides do not significantly interfere with secretion, expression yields and the independent folding of functional domains attached to them by flexible protease-resistant linkers. Modular gene cassettes encoding functional domains, linkers and multimerization domain can easily be combined into a cistron encoding the multimeric protein. Translation in a suitable host results in self-assembly to multimers larger than dimers. In cases in which one or both functional domains are not expressible in sufficient yields or fold into their native forms in the same expression host, multimeric proteins can be produced by manuf. of the subunits sep. by, e.g., in vitro translation, peptide synthesis and/or refolding and subsequently, e.g., chem. coupled to the remaining part of the multimeric protein. The use of these peptides is demonstrated by using them to build a tetramer of a single-chain anti-Ley **antibody** and a metal-binding domain using a tetramerization peptide derived from **p53**.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9637621	A2	19961128	WO 1996-EP2230	19960523
WO 9637621	A3	19970103		
W: CA, CN, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2222055	AA	19961128	CA 1996-2222055	19960523
EP 827544	A2	19980311	EP 1996-916159	19960523
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 11508126	T2	19990721	JP 1996-535396	19960523

L9 ANSWER 3 OF 20 MEDLINE
AN 97168950 MEDLINE
TI Characterization of scFv-421, a **single-chain antibody** targeted to **p53**.
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1997 Jan 13) 230 (2) 242-6.
Journal code: 0372516. ISSN: 0006-291X.
AU Jannot C B; Hynes N E
AB A gene encoding a **single-chain antibody** (scFv) which specifically binds the tumor suppressor protein **p53** has been constructed from RNA of hybridoma cells producing Pab 421. scFv-421 which was expressed and purified from bacteria specifically binds **p53**. scFv-421, as well as the previously described scFv-FRP5 and -R1R (1), were expressed intracellularly in mammalian cells and targeted to different subcellular locations, including the nucleus, cytoplasm, and endoplasmic reticulum (ER). High levels of all ER targeted scFv proteins, but not nuclear or cytoplasmic targeted proteins, were found in transfected COS-1 cells. In an attempt to stabilize the proteins, sequences encoding the mouse immunoglobin CK constant domain were added to each scFv construct. This led to a moderate increase in the cytoplasmic expression of scFv-FRP5.

L9 ANSWER 5 OF 20 MEDLINE
AN 1999039761 MEDLINE
TI Characterization of a new intrabody directed against the N-terminal region of human p53.
SO ONCOGENE, (1998 Nov 12) 17 (19) 2445-56.
Journal code: 8711562. ISSN: 0950-9232.
AU Cohen P A; Mani J C; Lane D P
AB Genes encoding the rearranged immunoglobulin heavy and light chain variable regions of DO-1, a monoclonal **antibody** directed against human **p53**, have been used to construct a **single-chain antibody**. DO-1 recognizes an N-terminal epitope in the region involved in the transactivation function of **p53** and the binding of Mdm2. The DO-1 single chain scFv expressed in the periplasm of E. coli or at the surface of the filamentous phage M13 retained the

immunological specificity and affinity of the full length **antibody** . Furthermore, the DO-1 recombinant **antibody** was able to inhibit the in vitro binding of Hdm2, and was shown to be a powerful protecting agent of **p53**'s DNA binding activity at 37 degrees C. The DO-1 **single-chain antibody** has been used to construct single-chain intracellular **antibodies** (intrabodies) for expression in the cytoplasm and the nucleus of mammalian cells. These anti-**p53** intrabodies were additionally modified by addition of a Ckappa domain to increase cytoplasmic and nuclear stability. Here we show that expression of the DO-1 **single-chain antibody** in the H1299 cell line results in an inhibition of **p53**'s transactivation function. The DO-1 intrabody is a useful tool to study those functions of **p53** driven by the N-terminal region of the protein.

L9 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2003 ACS
AN 1998:293531 CAPLUS
DN 129:3863
TI Anti-**p53 single-chain antibody** fragments and their uses
SO PCT Int. Appl., 54 pp.
CODEN: PIXXD2
IN Bracco, Laurent; Debussche, Laurent
AB The invention concerns **single-chain antibodies** directed against the **p53** protein, capable of being expressed in tumor cells, capable of restoring a DNA binding in vitro and a transcription activator function in vivo. The invention also concerns nucleic acids coding for these mols., the vectors contg. them and their uses.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9818825	A1	19980507	WO 1997-FR1921	19971027
W: AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GE, GH, HU, ID, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
FR 2755144	A1	19980430	FR 1996-13176	19961029
FR 2755144	B1	19981127		
AU 9749520	A1	19980522	AU 1997-49520	19971027
AU 745530	B2	20020321		
EP 941252	A1	19990915	EP 1997-912262	19971027
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, SI, FI				
BR 9712575	A	19991019	BR 1997-12575	19971027
JP 2001502553	T2	20010227	JP 1998-520119	19971027
ZA 9709738	A	19980522	ZA 1997-9738	19971029
NO 9901729	A	19990413	NO 1999-1729	19990413
KR 2000052845	A	20000825	KR 1999-703679	19990427

L9 ANSWER 7 OF 20 MEDLINE
AN 2000040151 MEDLINE
TI Ras and p53 intracellular targeting with recombinant single-chain Fv (scFv) fragments: a novel approach for cancer therapy?.
SO CANCER DETECTION AND PREVENTION, (1999) 23 (6) 506-10. Ref: 13
Journal code: 7704778. ISSN: 0361-090X.
AU Cochet O; Gruel N; Fridman W H; Teillaud J L
AB Intracellular expression of recombinant **antibodies** allows one to interfere with the functions of oncogenic molecules expressed in various cell compartments and has therefore a vast clinical potential in cancer therapy. We inhibited the functions of oncogenic Ras mutant forms by intracellular expression of a neutralizing **single-chain antibody** (scFv). In vitro studies indicated that the scFv is expressed in the cytosol of Xenopus laevis oocytes and of tumor cells, blocks ras-mediated activation processes, and induces tumor cell death. In vivo studies performed using scFv cDNA inserted into an adenoviral vector showed that the scFv dramatically affects tumor growth. Second, intracellular expression of scFvs directed against **p53** indicated

that these **antibody** fragments can be successfully targeted to cell nucleus, bind **p53**, and partially restore the transcriptional activity of **p53** mutants in human tumor cells. Thus, intracellular scFvs directed against oncogenic molecules may represent a new class of antitumor agents.

L9 ANSWER 8 OF 20 MEDLINE
AN 1999124403 MEDLINE
TI A tumor specific **single chain antibody** dependent gene expression system.
SO ONCOGENE, (1999 Jan 14) 18 (2) 559-64.
Journal code: 8711562. ISSN: 0950-9232.
AU Mary M N; Venot C; Caron de Fromentel C; Debussche L; Conseiller E; Cochet O; Gruel N; Teillaud J L; Schweighoffer F; Tocque B; Bracco L
AB The design of conditional gene expression systems restricted to given tissues or cellular types is an important issue of gene therapy. Systems based on the targeting of molecules characteristic of the pathological state of tissues would be of interest. We have developed a synthetic transcription factor by fusing a **single chain antibody** (scFv) directed against **p53** with the bacterial tetracycline repressor as a DNA binding domain. This hybrid protein binds to **p53** and can interact with a synthetic promoter containing tetracycline-operator sequences. Gene expression can now be specifically achieved in tumor cells harboring an endogenous mutant **p53** but not in a wild-type **p53** containing tumor cell line or in a non-transformed cell line. Thus, a functional transactivator centered on **single chain antibodies** can be expressed intracellularly and induce gene expression in a scFv-mediated specific manner. This novel class of transcriptional transactivators could be referred as 'trabodies' for transcription-activating-**antibodies**. The trabodies technology could be useful to any cell type in which a disease related protein could be the target of specific **antibodies**
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L9 ANSWER 12 OF 20 CAPLUS COPYRIGHT 2003 ACS
AN 1999:117846 CAPLUS
DN 130:295300
TI Restoration of transcriptional activity of p53 mutants in human tumor cells by intracellular expression of anti-p53 single chain Fv fragments
SO Oncogene (1999), 18(2), 551-557
CODEN: ONCNES; ISSN: 0950-9232
AU De Fromentel, Claude Caron; Gruel, Nadege; Venot, Corinne; Debussche, Laurent; Conseiller, Emmanuel; Dureuil, Christine; Teillaud, Jean-Luc; Tocque, Bruno; Bracco, Laurent
AB The authors report here the prodn. and the properties of single chain Fv fragments (scFvs) derived from the anti-**p53** monoclonal **antibodies** PAb421 and 11D3. 11D3 is a newly generated monoclonal **antibody** which exhibits properties very comparable to those of PAb421. The scFvs PAb421 and 11D3 are able to stably assoc. with **p53** and to restore the DNA binding activity of some **p53** mutants in vitro. When expressed in **p53**-/- human tumor cells, the scFv421 is essentially localized in the cytoplasm in the absence of **p53**, and in the nucleus when exogenous **p53** is present. Thus, **p53** is also able to stably assoc. with an anti-**p53** scFv in cells. Contransfection of **p53**-/- human tumor cells with expression vectors encoding the His273 **p53** mutant and either scFv leads to restoration of the **p53** mutant deficient transcriptional activity. These data demonstrate that, in human tumor cells, these scFvs are able to restore a function essential for the tumor suppressor activity of **p53** and may represent a novel class of mols. for **p53**-based cancer therapy.

L9 ANSWER 14 OF 20 CAPLUS COPYRIGHT 2003 ACS
AN 2000:608552 CAPLUS
DN 133:213074
TI Antibody fragment-targeted immunoliposomes for systemic gene delivery
SO PCT Int. Appl., 45 pp.
CODEN: PIXXD2
IN Xu, Liang; Huang, Cheng-Cheng; Alexander, William; Tang, Wenhua; Chang, Esther H.

AB Nucleic acid-immunoliposome compns. useful as therapeutic agents are disclosed. These compns. preferably comprise (i) cationic liposomes, (ii) a **single chain antibody** fragment which binds to a transferrin receptor, and (iii) a nucleic acid encoding a wild type **p53**. These compns. target cells which express transferrin receptors, e.g., cancer cells. These compns. can be used therapeutically to treat persons or animals who have cancer, e.g., head and neck cancer, breast cancer or prostate cancer.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000050008	A2	20000831	WO 2000-US4392	20000222
WO 2000050008	A3	20001221		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1154756	A2	20011121	EP 2000-915818	20000222

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

JP 2002537318	T2	20021105	JP 2000-600620	20000222

L9 ANSWER 15 OF 20 MEDLINE

AN 2001667808 MEDLINE

TI Systemic p53 gene therapy of cancer with immunolipoplexes targeted by anti-transferrin receptor scFv.

SO MOLECULAR MEDICINE, (2001 Oct) 7 (10) 723-34.
Journal code: 9501023. ISSN: 1076-1551.

AU Xu L; Tang W H; Huang C C; Alexander W; Xiang L M; Pirollo K F; Rait A; Chang E H

AB BACKGROUND: A long-standing goal in genetic therapy for cancer is a systemic gene delivery system that selectively targets tumor cells, including metastases. Here we describe a novel cationic immunolipoplex system that shows high in vivo gene transfer efficiency and anti- tumor efficacy when used for systemic **p53** gene therapy of cancer. MATERIALS AND METHODS: A cationic immunolipoplex incorporating a biosynthetically lipid-tagged, anti-transferrin receptor **single-chain antibody** (TfRscFv), was designed to target tumor cells both in vitro and in vivo. A human breast cancer metastasis model was employed to evaluate the in vivo efficacy of systemically administered, TfRscFv-immunolipoplex-mediated, **p53** gene therapy in combination with docetaxel. RESULTS: The TfRscFv-targeting cationic immunolipoplex had a size of 60-100 nm, showed enhanced tumor cell binding, and improved targeted gene delivery and transfection efficiencies, both in vitro and in vivo. The **p53** tumor suppressor gene was not only systemically delivered by the immunolipoplex to human tumor xenografts in nude mice but also functionally expressed. In the nude mouse breast cancer metastasis model, the combination of the **p53** gene delivered by the systemic administration of the TfRscFv-immunolipoplex and docetaxel resulted in significantly improved efficacy with prolonged survival. CONCLUSIONS: This is the first report using scFv-targeting immunolipoplexes for systemic gene therapy. The TfRscFv has a number of advantages over the transferrin (Tf) molecule itself: (1) scFv has a much smaller size than Tf producing a smaller immunolipoplex giving better penetration into solid tumors; (2) unlike Tf, the scFv is a recombinant protein, not a blood product; (3) large scale production and strict quality control of the recombinant scFv, as well as scFv-immunolipoplex, are feasible. The sensitization of tumors to chemotherapy by this tumor-targeted and efficient **p53** gene delivery method could lower the effective dose of the drug, correspondingly lessening the severe side effects, while decreasing the possibility of recurrence. Moreover, this approach is applicable to both primary and recurrent tumors, and more significantly, metastatic disease. The TfRscFv-targeting of cationic immunolipoplexes is a promising method of tumor targeted gene delivery that can be used for systemic gene therapy of cancer with the potential to critically impact the clinical management

of cancer.

L9 ANSWER 16 OF 20 MEDLINE
AN 2001610460 MEDLINE
TI **Single-chain antibody** against the common epitope of mutant **p53**: isolation and intracytosolic expression in mammalian cells.
SO JOURNAL OF IMMUNOLOGICAL METHODS, (2001 Dec 1) 258 (1-2) 169-81. Journal code: 1305440. ISSN: 0022-1759.
AU Govorko D; Cohen G; Solomon B
AB The peptide epitope FRHSVV is cryptic in wild-type **p53** and is exposed in many types of mutant **p53** molecules isolated from various tumors. Mutant **p53** marked by this epitope abrogates a tumor-suppressor function of wild-type **p53** and possibly contributes to the transforming potential of other oncogenic processes. We report here the construction of a single-chain scFv **antibody** gene library derived from the mRNA of a mouse immunized with the epitope peptide FRHSVV which mimics the common epitope in **p53** mutant protein molecules. The scFv was presented by phage display. The selected **antibody** gene, named ME1, was found to bind to the mutant **p53** protein but not to the wild-type **p53** protein. Preliminary studies show that the ME1 gene is expressed in the cytosol of mammalian cells. These findings suggest that the ME1 **single-chain antibody** may be useful as a tool for clarifying the role of mutant **p53** in tumor transformation, especially in cells heterozygous in **p53**, and possibly for gene therapy of tumors.

L9 ANSWER 17 OF 20 SCISEARCH COPYRIGHT 2003 ISI (R)
AN 2001:675058 SCISEARCH
TI Isolation of specific **single-chain antibodies** against **p53** from a fully synthetic library using two-hybrid system.
SO YEAST, (AUG 2001) Vol. 18, Supp. [1], pp. S299-S299. Publisher: JOHN WILEY & SONS LTD, BAFFINS LANE CHICHESTER, W SUSSEX PO19 1UD, ENGLAND. ISSN: 0749-503X.
AU Nery F C (Reprint); Ortega J M; Rodriguez M B

L9 ANSWER 18 OF 20 CAPLUS COPYRIGHT 2003 ACS
AN 2001:693461 CAPLUS
DN 135:256133
TI **Single chain antibody** against mutant **p53**
SO PCT Int. Appl., 46 pp.
CODEN: PIXXD2
IN Solomon, Beka; Cohen, Gerald; Govorko, Dimitri
AB More than 90 of mutations found in the **p53** protein produce a conformational change in **p53** which results in the exposure of an epitope, which is otherwise hidden in the hydrophobic core of the mol. A **single chain antibody** (scFv) which specifically recognizes this common mutant epitope in mutant **p53** but not in wild type **p53** is disclosed. Also described are a DNA mol. encoding the scFv, pharmaceutical compns. comprising the **antibody** and their use in methods of treatment.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001068801	A2	20010920	WO 2001-IL225	20010309
WO 2001068801	A3	20020207		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 2001039517	A5	20010924	AU 2001-39517	20010309
EP 1272609	A2	20030108	EP 2001-914142	20010309

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
US 2003022244 A1 20030130 US 2002-247488 20020920

L9 ANSWER 19 OF 20 MEDLINE
AN 2002727127 IN-PROCESS
TI Systemic tumor-targeted gene delivery by anti-transferrin receptor scFv-immunoliposomes.
SO Mol Cancer Ther, (2002 Mar) 1 (5) 337-46.
Journal code: 101132535. ISSN: 1535-7163.
AU Xu Liang; Huang Cheng-Cheng; Huang Weiqun; Tang Wen-Hua; Rait Antonina; Yin Yu Zhi; Cruz Idalia; Xiang Lai-Man; Pirollo Kathleen F; Chang Esther H
AB An ideal therapeutic for cancer would be one that selectively targets to tumor cells, is nontoxic to normal cells, and that could be systemically delivered, thereby reaching metastases as well as primary tumor. Immunoliposomes directed by monoclonal **antibody** or its fragments are promising vehicles for tumor-targeted drug delivery. However, there is currently very limited data on gene delivery using these vehicles. We have recently described a cationic immunoliposome system directed by a lipid-tagged, **single-chain antibody** Fv fragment (scFv) against the human transferrin receptor (TfR) that shows promising efficacy for systemic **p53** tumor suppressor gene therapy in a human breast cancer metastasis model. However, the extremely low yield of this lipid-tagged scFv limited further downstream development and studies. Here we report a different expression strategy for the anti-TfR scFv, which produces high levels of protein without any tags, and a different approach for complexing the targeting scFv to the liposomes. This approach entails covalently conjugating the scFv to the liposome via a cysteine at the 3'-end of the protein and a maleimide group on the liposome. Our results show that this conjugation does not impair the immunological activity or targeting ability of the scFv. The scFv-cys targets the cationic liposome-DNA complex (lipoplex) to tumor cells and enhances the transfection efficiencies both in vitro and in vivo in a variety of human tumor models. This scFv-immunoliposome can deliver the complexed gene systemically to tumors in vivo, where it is efficiently expressed. In comparison with the whole **antibody** or transferrin molecule itself, the scFv has a much smaller size for better penetration into solid tumors. It is also a recombinant protein rather than a blood product; thus, large scale production and strict quality control are feasible. This new approach provides a promising system for tumor-targeted gene delivery that may have potential for systemic gene therapy of various human cancers.

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L12 ANSWER 16 OF 26 CAPLUS COPYRIGHT 2003 ACS
AN 1996:733936 CAPLUS
DN 126:2476
TI Conditional gene expression system and its application to treatment of infections and cell hyperproliferation
SO PCT Int. Appl., 80 pp.
CODEN: PIXXD2
IN Bracco, Laurent; Schweighoffer, Fabien; Tocque, Bruno
AB A novel conditional gene expression system comprising creation and expression of bispecific chimeric proteins including a domain capable of selectively binding a given DNA sequence and a sensing domain capable of specifically binding a transactivator or transrepressor or a transactivator or transrepressor complex is claimed. The chimeric protein may contain TetR or Cro fused to an oligomerization domain of **p53**, STAT, or NF.kappa.B or to an antibody or antibody fragment (e.g., an **scFv**) which binds to a transactivator. The transactivator may be supplied by an infecting agent, e.g., protein tat of HIV, proteins E6/E7 of papillomavirus, or protein EBNA of Epstein-Barr virus. The target of this chimeric protein-transactivator complex is a chimeric gene comprising a TetR/Cro-binding operator and transactivator-binding promoter linked to a gene encoding a therapeutic protein such as diphtheria toxin, ricin A, or cytosine deaminase. The system was demonstrated in human osteosarcoma cells SAOS-2 which are deficient in **p53**. Expression of tet operator-linked luciferase gene was stimulated when a chimeric TetR-**p53** oligomerization domain protein was coexpressed with wild-type **p53**.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9630512	A1	19961003	WO 1996-FR477	19960329
W: AL, AU, BB, BG, BR, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KP, KR, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
FR 2732348	A1	19961004	FR 1995-3841	19950331
FR 2732348	B1	19970430		
ZA 9602506	A	19961001	ZA 1996-2506	19960328
CA 2214451	AA	19961003	CA 1996-2214451	19960329
AU 9654020	A1	19961016	AU 1996-54020	19960329
AU 716748	B2	20000302		
EP 817845	A1	19980114	EP 1996-911000	19960329
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, SI, FI				
BR 9607928	A	19980609	BR 1996-7928	19960329
JP 11503011	T2	19990323	JP 1996-529022	19960329
NO 9704449	A	19970926	NO 1997-4449	19970926

L22 ANSWER 8 OF 10 MEDLINE
AN 86281845 MEDLINE
TI Monoclonal antibody analysis of p53 expression in normal and transformed cells.
SO JOURNAL OF VIROLOGY, **(1986 Aug)** 59 (2) 444-52.
Journal code: 0113724. ISSN: 0022-538X.
AU Yewdell J W; Gannon J V; Lane D P
AB The cellular phosphoprotein p53 binds tightly and specifically to simian virus 40 T antigen and the 58,000-molecular-weight adenovirus E1b protein. Many human and murine tumor cell lines contain elevated levels of the p53 protein even in the absence of these associated viral proteins. Recently the cloned p53 gene, linked to strong viral promoters, has been shown to complement activated ras genes in transformation of primary rodent cell cultures. Overexpression of the p53 gene alone rescues some primary rodent cell cultures from senescence. We **isolated** three new monoclonal antibodies to the p53 protein, designated PAb242, PAb246, and PAb248, and mapped the epitopes they recognized on p53 in comparison with other previously **isolated** antibodies. At least five sterically separate epitopes were defined on murine p53. One of the antibodies, PAb246, recognizes an epitope on p53 that is unstable in the absence of bound simian virus 40 T antigen. This effect is demonstrable in vivo and in newly developed in vitro assays of T-p53 complex formation. Using the panel of **anti-p53 antibodies** and sensitive immunocytochemical methods, we found that p53 has a predominantly nuclear location in established but not transformed cells as well as in the vast majority of transformed cell lines. Several monoclonal antibodies to p53 showed cross-reactions with non-p53 components in immunocytochemical staining.

L25 ANSWER 1 OF 3032 CAPLUS COPYRIGHT 2003 ACS
AN 1986:528643 CAPLUS
DN 105:128643
TI Isolation of human-**p53**-specific **monoclonal antibodies** and their use in the studies of human **p53** expression
SO European Journal of Biochemistry (**1986**), 159(3), 529-34
CODEN: EJBCAI; ISSN: 0014-2956
AU Banks, Lawrence; Matlashewski, Greg; Crawford, Lionel
AB The isolation and construction of a complete human phosphoprotein **p53** cDNA and subsequent expression in monkey cells is described. A set of new anti-(human **p53**) **monoclonal antibodies** was obtained and used to show the expression of the human **p53** cDNA in COS-1 cells. These **antibodies** enable the specific detection of human **p53**, which is synthesized in the presence of **p53** from other species. Fusion proteins of **p53** with .beta.-galactosidase were used firstly as antigen and secondly, in conjunction with competition assays, to localize the determinants recognized by the **antibodies**. At least 2 previously unrecognized epitopes are involved and 2 of the **antibodies** are human-**p53**-specific. The epitopes are denaturation-resistant and the **antibodies** are, therefore, valuable for immunoblotting as well as immunopptn. and enzyme-linted immunoassay. Transfection of plasmids contg. complete human **p53** cDNA into monkey (COS-1) cells cause expression of human **p53** recognized by the **monoclonal antibodies**. Control plasmids did not induce immunoreactive protein.

L25 ANSWER 2 OF 3032 CAPLUS COPYRIGHT 2003 ACS
AN 1995:251852 CAPLUS
DN 122:312500
TI Mutations in **p53** produce a common conformational effect that can be detected with a panel of **monoclonal antibodies** directed toward the central part of the **p53** protein
SO Oncogene (**1994**), 9(12), 3689-94
CODEN: ONCNES; ISSN: 0950-9232
AU Legros, Yann; Meyer, Aurelia; Ory, Katherine; Soussi, Thierry
AB Human **p53** displays two immunodominant regions localized in the amino and carboxy termini of the protein. Using a truncated **p53** (resides 66 to 361), the authors selected eight new **monoclonal antibodies** directed to the central part of the protein. The authors identified the epitopes recognized by seven out of eight **antibodies** with a set of overlapping peptides. One of these **antibodies** had an epitope similar to PAb240, whereas the others recognized novel and diverse antigenic determinants. Using a series of 19 **p53** mutants, the authors show that the behavior of several of the new **monoclonal antibodies** is similar to that of PAb240 despite their various epitope localizations. This suggests that different mutations in the **p53** protein induce an overall conformational change that can be detected by various **monoclonal antibodies** directed toward the central part of the protein.

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FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED AT 15:02:01 ON 25 FEB 2003

L1 4 S 11D3 (L) ANTIBOD?

L2 1 DUP REM L1 (3 DUPLICATES REMOVED)

=> d an ti so au ab l2 1

L2 ANSWER 1 OF 1 MEDLINE DUPLICATE 1

AN 1999124402 MEDLINE

TI Restoration of transcriptional activity of p53 mutants in human tumour cells by intracellular expression of anti-p53 single chain Fv fragments.

SO ONCOGENE, (1999 Jan 14) 18 (2) 551-7.
Journal code: 8711562. ISSN: 0950-9232.

AU Caron de Fromentel C; Gruel N; Venot C; Debussche L; Conseiller E; Dureuil C; Teillaud J L; Tocque B; Bracco L

AB We report here the production and the properties of single chain Fv fragments (scFvs) derived from the anti-p53 monoclonal **antibodies** PAb421 and **11D3**. **11D3** is a newly generated monoclonal **antibody** which exhibits properties very comparable to those of PAb421. The scFvs PAb421 and **11D3** are able to stably associate with p53 and to restore the DNA binding activity of some p53 mutants in vitro. When expressed in p53 -/-human tumour cells, the scFv421 is essentially localized in the cytoplasm in the absence of p53, and in the nucleus when exogenous p53 is present. Thus, p53 is also able to stably associate with an anti-p53 scFv in cells. Cotransfection of p53 -/- human tumour cells with expression vectors encoding the His273 p53 mutant and either scFv leads to restoration of the p53 mutant deficient transcriptional activity. These data demonstrate that, in human tumour cells, these scFvs are able to restore a function essential for the tumour suppressor activity of p53 and may represent a novel class of molecules for p53-based cancer therapy.

27 ANSWER 3 OF 2318 CAPLUS COPYRIGHT 2003 ACS
AN 1990:418807 CAPLUS
DN 113:18807
TI Activating mutations in **p53** produce a common conformational effect. A **monoclonal antibody** specific for the mutant form
SO EMBO Journal (**1990**), 9(5), 1595-602
CODEN: EMJODG; ISSN: 0261-4189
AU Gannon, J. V.; Greaves, R.; Iggo, R.; Lane, D. P.
AB Point mutations in the **p53 gene** are the most frequently identified genetic change in human cancer. They convert murine **p53** from a tumor suppressor **gene** into a dominant transforming oncogene able to immortalize primary cells and bring about full transformation in combination with an activated ras **gene**. In both the human and murine systems, the mutations lie in regions of **p53** conserved from man to Xenopus. A **monoclonal antibody** to **p53** designated PAb240 which does not immunoppt. wild-type **p53** was developed. A series of different **p53** mutants all react more strongly with PAb240 than with PAb246. The PAb240-reactive form of **p53** cannot bind to SV40 large T antigen but does bind to HSP70. In contrast, the PAb246 form binds to T antigen but not to HSP70. PAb240 recognizes all forms of **p53** when they are denatured. It reacts with all mammalian **p53** and chicken **p53** in immunoblots. It is proposed that immunopptn. of **p53** by PAb240 is diagnostic of mutation in both murine and human systems and suggest that the different point mutations which convert **p53** from a recessive to a dominant oncogene exert a common conformational effect on the protein. This conformational change abolishes T antigen binding and promotes self-oligomerization. These results are consistent with a dominant neg. model where mutant **p53** protein binds to and neutralizes the activity of **p53** in the wild-type conformation.